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Description

The present invention is concerned with cell viability assays and, more particularly but not exclusively, methods and apparatus for conducting cytotoxicity assays on biopsied cells.

It is known that cancerous or otherwise abnormal cells of identical histopathological type show a wide range of responsiveness to particular drug therapies among individual patients. Predictive techniques, similar to the culture and sensitivity assays used for the management of microbial infections, would be of great assistance in selecting effective chemotherapy for individual cases.

Without individualized anti-cancer drug regimens, practitioners are forced to rely on past experience or reports on similar cell disorders or trial-and-error proceedures. With the increasing number of anti-cancer agents available and the limited time often available for modifying doses or agents, the task of selecting the optimal regimen, without the aid of predictive assays, is very difficult.

A number of predictive systems have been proposed. See, for example, Salmon et al., "Quantitation of Differential Sensitivity of Human Stem Cells to Anti-cancer Drugs", Vol. 298 New England Journal of Medicine pp. 1321-1327 (1978). Typically, the prior art techniques involve the cloning of single cell suspensions from biopsy specimens in soft agar after brief exposure to particular anti-cancer drugs. See also, Buick et al., "Development of an Agar-Methyl Cellulose Clonogenic Assay for Cells in Transitional Cell Carcinoma of the Human Bladder", Vol. 39 Cancer Research pp. 5051-5056 (1979) and Von Hoff et al., "Direct Cloning of Human Malignant Malanoma in Soft Agar Culture", Vol. 50 Cancer pp. 695-701 (1982), for further details on agar culture techniques.

Various difficulties limit the usefulness of agar culture studies for predicting the effectiveness of cytotoxic agents against abnormal cells. Only a small fraction of biopsied cancer cells grow in soft agar. For example, when cell suspensions from myeloma specimens are plated in agar, plating efficiencies of 1:1000 are not uncommon. Thus, for statistically significant results comparing different drugs at different doses, large numbers of cells are required. In practice, only 60% of the patients have tumors large enough to be assayed. It is also not certain that colonies formed in agar will be derived from the most malignant tumor cells. Moreover, agar techniques typically limit drug exposure to a relatively brief period (i.e. one hour) prior to plating while the cell is suspended in a physiological solution. Hence, neither the exposure technique nor the subsequent growth in agar accurately mimic in vivo conditions. Additionally, the time required for evaluation is long (i.e. 14 to 30 days) compared to the often urgent need to establish a protocol for therapy. Finally, measurements of drug sensitivity by counting cell colonies can be subjective, statistically inaccurate and time consuming.

Another predictive system which has been proposed for chemotherapy studies involves the use of cell cultures grown in an artificial organ made of a matrix of synthetic capillaries. Quartles et al., "Hemodialysis-Matrix Perfusion Culture System: A New Technique for Studying Chemotherapeutic Activity Tumor Cells", Vol. 16 in vitro 246 (1980), report the effect of one anti-cancer agent on tumor cells grown in an artificial capillary system. Following exposure to the drug, the cultured cells were removed from the capillary matrix and assayed for total and viable cells, as well as colony forming ability and growth in soft agar. For a review of capillary cultures, generally, see Schratter, "Cell Culture with Synthetic Capillaries", Vol. XIV Methods in Cell Biology pp. 95-103 (1976).

The capillary technique for studying chemotherapeutic activity reported by Quartles, supra, is subject to many of the same problems that limit the usefulness of agar studies. Quartles and his co-workers had to remove the cells from the capillary system in order to count total and viable cells. In practice, removing cells without damage from a capillary matrix is an arduous task. Typically, the cells are removed from the capillary matrix by enzyme treatment but this treatment can be more effective on dead cells than on living cells and quantitative results are difficult to obtain. Moreover, the culture is lost after enzyme treatment and cannot be used again.

Additionally, capillary systems also suffer from a need for large quantities of tumor cells. Typically, assays using capillary systems required on the order of 5 million cells per vessel. To perform a concurrent battery of cytotoxicity assays, a half dozen or more capillary vessels (i.e. 30 million cells) must be employed. Obtaining sufficient tumor cells for these purposes from biopsy samples directly is not possible and hence an analyst wishing to undertake a comprehensive toxicity study must first grow the biopsy sample into a larger colony. Growing such a colony or colonies may not be feasible or can take months, severely hampering any predictive use of the assay.

Accordingly, there exists a need for better apparatus and methods for performing cytotoxicity assays. An assay apparatus that would operate on smaller-sized samples, for example, of the order of 100,000 cells or less, and accurately predict cytotoxicity, would find practical use as a predictive measure of drug sensitivity and would satisfy a long-felt need in the field of cancer treatment.

Moreover, a predictive culture system should

be easily inoculated and cell growth as well as drug exposure should mimic closely the human environment. Also importantly, the drug sensitivity should be quantifiable by a simple and accurate method in a relatively short time and preferably in a way that would permit a clinician to obtain a reading on the effectiveness of a particular agent without destruction of the culture so that the effects of a multi-step protocol (i.e. varying in agents or doses) can be measured sequentially.

In International Patent Application No. W084/03 047 (EP.84 90 0685.3), the present applicant has disclosed a simple, sensitive, cytotoxicity assay capable of widespread clinical application. In the method disclosed therein the number of living cells in a culture vessel is evaluated by measuring the retention of fluorescein or a similar label by the cell membranes. The cultured cells are allowed to accumulate fluorescein through fluorochromasia, which occurs when a fluorogenic substrate, typically a nonpolar fluorogenic substrate such as an ester of fluorescein and an aliphatic acid, is introduced into a cell culture. The fluorogenic substrate penetrates the cell membranes where it is enzymatically hydrolyzed, liberating fluorescein and staining the cell brightly fluorescent under blue light. Since fluorescein, a negatively charged molecule, does not diffuse readily across the cytoplasmic membrane of normal cells, the process causes intracellular accumulation of fluorescein. However, when a dead cell is treated with the fluorogenic substrate, no intracellular accumulation of fluorescein is observed. Therefore, if the cells in the vessel have been killed by an agent prior to the introduction of a fluorescent substrate, hydrolysis of the substrate will not result in intracellular fluorescein accumulation.

Changes in fluorescence may be measured by monitoring the fluorescent activity of fluids eluted from the culture vessel which can be provided with at least one sampling port for this purpose; or the fluorescence within the vessel may be observed.

It was disclosed in said W084/03047, which forms part of the state of the art under Article 54(3) EPC only, that readings of fluorescence in samples taken directly from the cell compartment may be up to four times stronger than samples taken from the return stream of the perfusion network.

It was not however disclosed that advantageously changes in fluorescence could be measured by direct photometric observation of the cells whilst they remain in the environment within the culture vessel.

The present invention provides a method of assaying the viability of animal cells to a particular culture environment provided in a culture vessel comprising the steps of: (a) providing the cells in said culture environment; (b) culturing the cells by perfusion of a nutrient into said environment; (c) contacting the cells with a fluorogenic substrate, whereby living cells accumulate a characteristic amount of fluoroscence; and (d) measuring changes in fluorescence as an indication of the viability of the cells; characterised in that the changes in fluorescence are measured by a direct photometric observation of the cells whilst they remain in the environment within the culture vessel.

The invention also provides a method of assaying sensitivity of biopsied cells to cytotoxic agents comprising the steps of: (a) providing the cells in a culture environment provided in a culture vessel; (b) culturing the cells by perfusion of a nutrient into said environment; (c) contacting the cells with a fluorogenic substrate, whereby living cells accumulate a characteristic amount of fluorescence; (d) contacting the cells with the cytotoxic agent; and (e) measuring changes in fluorescence as an indication of the sensitivity of the cells to the cytotoxic agent; characterised in that the changes in fluorescence are measured by a direct photometric observation of the cells whilst they remain in the environment within the vessel.

The invention also provides apparatus constructed and arranged to carry out a method according to the invention comprising (a) a culture vessel divided into a cell-containing zone and a nutrient-containing zone by a fluid-permeable perfusion barrier; (b) a source of fluorogenic substrate in communication with cell-containing zone; and (c) a fluorimeter arranged to measure the fluorescence accumulated by the living cells; characterised in that the fluorimeter is positioned to measure changes in fluorescence by a direct photometric observation of the cells in the culture vessel.

The fluorimeter comprises for example a photographic or video camera positioned for direct photometric observation of the cells.

An embodiment of the invention comprises a vessel including at least one cell growth surface and is preferably structured so that the vessel may be inoculated with undissociated fragments of biopsied tissues, thus retaining the basic cellular composition of the tumor (many tumors have been shown to exhibit cellular heterogeneity). The perfusion of oxygen and nutrients combined with removal of cellular waste as well as the three-dimensional structure of the vessel make it likely that even tumor cells unable to grow in agar can be kept viable in the apparatus. Moreover, the sensitivity of fluorescence detection techniques allows the vessel to be employed with small numbers of cells, thus permitting simultaneous studies from even biopsy specimens of limited size, such as colon or lung cancer biopsies.

In one preferred embodiment, the vessel is formed by an upper and a lower body element

which are clamped together to form a shell defining a cavity for cell inoculation and nutrient circulation. The cells, themselves, are secured within the cavity by a cylindrical compartment bounded by an upper and a lower membrane, which are preferably a porous material such as fibrous interwoven cellulose or a polymeric mesh. The cell compartment, membranes and shell can be sealed together by O-ring gaskets. Ports for cell inoculation and fluid passage also are disposed within the vessel.

A system and protocol are provided for performing cytotoxicity studies including apparatus and methods for culturing the biopsied cells, for providing oxygenated nutrients, for introducing a fluorogenic substrate, for introducing anti-cancer agents and for measuring the released fluorescence. Cytotoxicity is determined by measuring fluorescence by direct photometric comparisons of the cells in the vessel before and after exposure to the anti-cancer agent.

In one method, the biopsy sample is mechanically teased into smaller fragments and suspended in a medium to separate most of the normal cells from the malignant cells. The tumor cells can be further purified by density gradient centrifuging.

The tumor aggregates are then inoculated into the vessel and, after culturing in the vessel with nutrient medium, the fluorogenic substrate is introduced and the resulting fluorescein is allowed to accumulate. Once a steady state condition is reached, the excess substrate is removed and normal nutrient perfusion continues. The therapeutic agent to be tested can then be introduced into the cell compartment (directly or by perfusion), and monitoring carried out for a period of time (i.e. about 15 minutes to 2 hours) for changes in the amount of fluorescein released. With agents exhibiting delayed (i.e. radiomimetic) effects, more detailed records of the kinetics and fluorescence of the cell culture or its efflux can be obtained following each of a series of perfusions with fluorogenic substrate. Different fluorogenic substrates and temperature shifting can be employed to obtain more rapid or slower results.

The vessel is constructed with a transparent viewing port and the changes in fluorescence measured directly by photometric analysis. In one illustrated embodiment, the cell compartment is illuminated by monochromatic light and then photographed through a matching light filter to capture only the fluorescence of the living cells. The optical density of the photograph negative provides a simple, accurate measure of the intracellular fluorescein and, therefore, a parameter for cytotoxic effects of the agent being tested.

The methods disclosed herein, permit the clinician to test the effects of prolonged exposure, different combinations and programmed schedules of drugs. Additionally, if a drug gives a negative cytotoxicity test, the vessel and culture can be recycled for subsequent drug testing.

The various therapeutic or chemical agents which can be tested for effectiveness on individual cell cultures include: adriamycins, mitomycins, actinomycins, neomycins, vincristine, vinblastine, chlorambucil, cis-platinum, 6-mercapto purine, methotrexate, cyclophosphamide, melphalen, carmustine, methyl ester DOPA, BCNU, DTIC, 5-fluoruracil, m-AMSA, mitoxantrone, methyl GAG, acivicin, thymidine, hormones, antibodies, prostaglandins and lymphokines as well as X-rays or other agents as they become available.

A wide variety of commercially available growth media from companies such as Gibco Corporation and others may be employed as nutrients. These media are sold under names such as Dulbecco's Modified Eagle Medium (DMEM), Roswell Park Medium (RPMI) and Minimal Eagle Medium (MEM) and typically consist of amino acids, salts, vitamins, blood serum and other nutrients. Alternatively, in clinical applications, it may be preferred to use serum from the biopsied patient for all or part of the growth medium in order to further mimic in vivo exposure to the agents undergoing testing. Other additions can include X-irradiated feeder cells from autologous or heterologous origin, soft agar, and plasma clots.

Moreover, while a primary objective is to present methods and apparatus for predicting the responsiveness of cancer cells to chemotherapeutic agents, other uses may also prove valuable. For examples, drugs against other cell abnormalities can be tested and the methods and apparatus can also be used in assessing the effects of drugs on non-cancerous cells as a measure of the side-effects that a particular course of chemotherapy would cause in the patient.

There now follows a description, to be read with reference to the accompanying drawings of apparatus and methods embodying the invention. This description is given by way of example only, and not by way of limitation of the invention.

In the accompanying drawings:

Fig. 1 is an exploded perspective view of a cell culturing apparatus embodying the present invention;

Fig. 2 has been cancelled;

Fig. 3 is a schematic top cross-sectional view of the apparatus;

Fig. 4 is a perspective view of the apparatus;

Fig. 5 is a schematic side cross-sectional view of the apparatus;

Fig. 6 is a schematic drawing of a cytotoxicity assay system employing the apparatus; and

Figures 7a and 7b are photographs showing assay results.

In Fig. 1 a culture vessel 10 is shown having an upper shell element 12 and a lower shell element 14 adapted to be clamped together by nuts 16 and bolts 18a. Within the cavity of the shell elements 12, 14, a cell compartment 20 is formed by a hollow cylindrical element 18 and porous upper and lower membranes 22, 24, respectively. The edges of the membranes 22, 24, and the hollow cylindrical element 18 are sealed to the shell by upper and lower elastomeric O-ring gaskets 26, 28, respectively.

As shown in Fig. 1 and in more detail in the cross-sectional view of Fig. 2, inlet 30 and outlet 32 in the lower shell element 14 permit the passage of nutrient-carrying fluids into and out of the cavity. Outlet 34 in the upper shell element permits venting of air from the cavity and can also serve as an alternative outlet for the nutrient medium. Ports 36 and 38 in the hollow cylindrical element 18 are connected to tubes 40 and 42, respectively which pass through slots 44 in the lower shell element 14 when the vessel 10 is clamped together. The ports 36, 38 serve as direct access points to the cell compartment 20 and are typically covered by septum plugs (not shown) when not in use. The upper surface 46 of the cavity 48 is preferably concave or conical to aid in venting.

A top cross-sectional view of the vessel 10 is presented in Fig. 3 showing the upper membrane 22 which defines the top of the cell compartment 20. In one preferred embodiment, the porous membrane 22 can be matted or woven fibrous cellulose, such as the material from which tea bags are made. Alternatively, filter paper or synthetic meshes such as woven nylon, cellulose acetate or silicon polycarbonate may be employed. The pore size can range from 5 to 35 micrometers, preferably 10-30 microns and more preferably 15-25 micrometers, when non-disassociated biopsy fragments are used for inoculation. However, when single cell suspensions are used, a smaller pore size is preferred, for example, of the order of 5 micrometres or less. When synthetic meshes are employed as the membranes 22, 24 in vessel 10 it can be advantageous to coat the membranes with a material such as agar, collagen, fibronectin or gelatin and/or soak them overnight in serum before assembly to ensure better compatibility with the inoculated biopsy cells. Nutrient perfusion is accomplished by a peristaltic pump or gravity flow.

The culture vessel 10 has a transparent viewing window 52 in the upper shell element 12.

In Fig. 6 a system 60 employing the vessel 10 is shown having a light source 62, preferably of blue monochromatic light, which illuminates vessel 50 through window 52. Light from the vessel 50 passes through filter 64, which is preferably a matching blue filter and absorbs the blue wavelengths while passing only the greenish fluorescent light. The fluorescence is captured by camera 66 mounted on a low-power microscope and processed by light density meter 68 to yield cell viability measurements at each step of the assay procedure. Camera 66 may take still photographs, the negatives of which are analysed by the light density meter 68, or alternatively, camera 66 and meter 68 may be formed as a high speed video scanning and electronic processing device for fully-automated operations. Figs. 7a and 7b show the results of an assay employing the system of Fig. 6. In addition, computer scanning of photographs provides parameters with which to evaluate objectively cell proliferation or death.

A typical protocol for conducting a cytotoxicity assay embodying the present invention begins with the straining of a biopsy sample through a 16 gauge stainless steel mesh to obtain suitable fragments for inoculation into the vessel. Enzyme extraction and single cell suspensions need not be employed. The straining process can be repeated as necessary until sample fragments roughly of the order of 0.2 mm^3 are obtained.

The fragments are then decanted in a medium, such as RPMI-1640 medium, for about 5 minutes at 0^oC wherein non-aggregated normal cells (i.e. red blood cells, lymphocytes, macrophages) will form a supernatant suspension while the aggregates of malignant cells can be collected in a sediment layer. The decantation procedure is repeated two more times. Typically, the deposited material comprises about 80-90% tumor cells. The sedimented cell fragments can be further purified, if desired, by layering the cell suspension on a solution of albumin or similar specific gravity carbohydrate solution and centrifuging, for example at about 20-300 x G for about 5 minutes. Under these conditions, the purified tumor cell aggregates separate at the interface and can be drawn off the solution.

The biopsy fragments are mixed again with a medium, such as the RPMI medium, and inoculated into the cell compartment 20 of vessel 10 via a syringe through the septum plug on one or the other ports 36, 38. Inside the cell compartment membranes 22, 24 (typically filter paper of interlaced cellulose fibers with pore sizes of roughly 20 micrometers on the average) secure the tumor aggregates within the compartment while providing passageways for the nutrient medium to diffuse through the vessel.

After an appropriate culturing period (typically 1-10 days) the fluorescent substrate can be applied either by injection directly into the cell compartment through one or both ports or, preferably, by mixture into the circulating medium. (The fluorogenic substrate can be substituted for the calf

serum in the medium). Exposure to the substrate typically lasts from 15 minutes to 2 hours, preferably one-half hour at room temperature.

After exposure, the circulating medium containing the substrate is replaced with a new medium, preferably including a serum component. There is normally no need to wash the cell compartment to remove excess substrate. The cell compartment, itself, is then examined to detect fluorescence levels and thereby determine the status of the cells. This procedure can be repeated daily to determine long-term effects. An adjustment period of about 24 hours is preferred before introducing the therapeutical agent to be tested.

The therapeutic agent is typically introduced by adding it to the circulating medium thereby mimicking the method by which the patient would be exposed. Following exposure another adjustment period of about 24 hours is preferred before the culture is again exposed to the fluorogenic substrate.

It will be realized that cytotoxicity can be determined by measure fluorescence in the efflux of the vessel concomitantly with the direct photometric comparisons of the cells in the vessel before and after exposure to the cytotoxic agent.

Claims

1. A method of assaying the viability of animal cells to a particular culture environment provided in a culture vessel comprising the steps of: (a) providing the cells in said culture environment; (b) culturing the cells by perfusion of a nutrient into said environment; (c) contacting the cells with a fluorogenic substrate, whereby living cells accumulate a characteristic amount of fluorescence; and (d) measuring changes in fluorescence as an indication of the viability of the cells; characterised in that the changes in fluorescence are measured by a direct photometric observation of the cells whilst they remain in the environment within the culture vessel.

2. A method of assaying sensitivity of biopsied cells to cytotoxic agents comprising the steps of: (a) providing the cells in a culture environment provided in a culture vessel; (b) culturing the cells by perfusion of a nutrient into said environment; (c) contacting the cells with a fluorogenic substrate, whereby living cells accumulate a characteristic amount of fluorescence; (d) contacting the cells with the cytotoxic agent; and (e) measuring changes in fluorescence as an indication of the sensitivity of the cells to the cytotoxic agent; characterised in that the changes in fluorescence are measured by a direct photometric observation of the cells whilst they remain in the environment within the vessel.

3. A method according to claim 2, wherein the biopsied cells are prepared by dissociation of a biopsy sample into fragments and effecting a separation between any malignant cellular material and normal cellular material by decantation.

4. A method according to claim 3, wherein the sample is mechanically dissociated into fragments.

5. A method according to claim 3 or claim 4, wherein the sample is dissociated into fragments of the order of 0.2 cubic millimeters in size.

6. A method according to any one of claims 2 to 5, wherein the cells are contacted with the cytotoxic agent by perfusion.

7. A method according to any one of the preceding claims, wherein the changes in fluorescence are photometrically recorded by a photographic or video camera.

8. A method according to any one of the preceding claims wherein the photometric observation is conducted with the culture illuminated by blue monochromatic light.

9. A method according to claim 7, wherein the photometric observation includes filtering light from the culture with a matching blue filter.

10. A method according to any one of the preceding claims wherein the direct photometric observation of cells is through a transparent wall surface of the vessel.

11. A method according to any one of the preceding claims wherein the perfusion takes place by means of a fluid permeable membrane.

12. A method according to claim 11, wherein the fluid permeable membrane has a pore size from 5 to 35 microns.

13. A method according to claim 11, wherein the fluid permeable membrane comprises a synthetic material.

14. A method according to claim 11. wherein the fluid permeable membrane comprises a fibrous cellulose material.

15. A method according to claim 11, wherein the fluid permeable membrane comprises a polymeric mesh.

16. A method according to claim 11, wherein two opposite sides of the vessel are defined by fluid permeable membranes through which the perfusion takes place.

17. Apparatus constructed and arranged to carry out a method according to any one of the preceding claims comprising (a) a culture vessel divided into a cell-containing zone and a nutrient-containing zone by a fluid-permeable perfusion barrier; (b) a source of fluorogenic substrate in communication with cell-containing zone; and (c) a fluorimeter arranged to measure the fluorescence accumulated by the living cells; characterised in that the fluorimeter is positioned to measure changes in fluorescence by a direct photometric observation of the cells in the culture vessel.

18. Apparatus according to claim 17, wherein the fluorimeter comprises a photographic or video camera positioned for direct photometric observation of the cells.

Revendications

1. Procédé de détermination de la viabilité de cellules animales dans un environnement de culture particulier créé dans un récipient de culture, comprenant les étapes consistant: (a) à placer les cellules dans ledit environnement de culture; (b) à cultiver les cellules par perfusion d'une substance nutritive dans ledit environnement; (c) à mettre les cellules en contact avec un substrat fluorogène, de telle sorte que les cellules vivantes accumulent une quantité caractéristique de fluorescence; et (d) à mesurer les modifications de fluorescence en tant qu'indication de la viabilité des cellules, caractérisé en ce que les modifications de fluorescence sont mesurées par une observation photométrique directe des cellules tandis qu'elles restent dans l'environnement à l'intérieur du récipient de culture.

2. Procédé de détermination de la sensibilité de cellules animales biopsiées à des agents cytotoxiques, comprenant les étapes consistant: (a) à placer les cellules dans un environnement de culture créé dans un récipient de culture; (b) à cultiver les cellules par perfusion d'une substance nutritive dans ledit environnement; (c) à mettre les cellules en contact avec un substrat fluorogène, de telle sorte que les cellules vivantes accumulent une quantité caractéristique de fluorescence; (d) à mettre les cellules en contact avec l'agent cytotoxique; et (e) à mesurer les modifications de fluorescence en tant qu'indication de la sensibilité des cellules à l'agent cytotoxique, caractérisé en ce que les modifications de fluorescence sont mesurées par une observation photométrique directe des cellules tandis qu'elles restent dans l'environnement à l'intérieur du récipient de culture.

3. Procédé selon la revendication 2, dans lequel les cellules biopsiées sont préparées par dissociation d'un échantillon de biopsie en fragments et en effectuant une separation entre un matériel cellulaire malin et le matériel cellulaire normal par décantation.

4. Procédé selon la revendication 3, dans lequel l'échantillon est dissocié mécaniquement en fragments.

5. Procédé selon la revendication 3 ou 4, dans lequel l'échantillon est dissocié en fragments dont la taille est de l'ordre de 0,2 mm^3.

6. Procédé selon l'une quelconque des revendications 2 à 5, dans lequel les cellules sont mises en contact avec l'agent cytotoxique par perfusion.

7. Procédé selon l'une quelconque des revendications 1 à 6, dans lequel les modifications de fluorescence sont enregistrées photométriquement par un appareil photographique ou une caméra vidéo.

8. Procédé selon l'une quelconque des revendications 1 à 7, dans lequel l'observation photométrique est conduite en éclairant la culture par une lumière monochromatique bleue.

9. Procédé selon la revendication 7, dans lequel l'observation photométrique comprend la filtration de la lumière issue de la culture avec un filtre bleu adapté.

10. Procédé selon l'une quelconque des revendications 1 à 9, dans lequel l'observation photométrique directe des cellules est effectuée à travers une surface de paroi transparente du récipient.

11. Procédé selon l'une quelconque des revendications 1 à 10, dans lequel la perfusion se produit au moyen d'une membrane perméable aux fluides.

12. Procédé selon la revendication 11, dans lequel la membrane perméable aux fluides a une grosseur de pores de 5 à 35 µm.

13. Procédé selon la revendication 11, dans lequel la membrane perméable aux fluides est faite d'une matière synthétique.

14. Procédé selon la revendication 11, dans lequel la membrane perméable aux fluides est faite d'une matière cellulosique fibreuse.

15. Procédé selon la revendication 11, dans lequel la membrane perméable aux fluides est un tissu à mailles de polymère.

16. Procédé selon la revendication 11, dans lequel deux côtés opposés du récipient sont définis par des membranes perméables aux fluides, à travers lesquelles se produit la perfusion.

17. Appareil construit et agencé pour l'exécution d'un procédé selon l'une quelconque des revendications 1 à 16, comprenant (a) un récipient de culture divisé en une zone contenant les cellules et en une zone contenant la substance nutritive par une barrière de perfusion perméable aux fluides; (b) une source de substrat fluorogène en communication avec la zone contenant les cellules; et (c) un fluorimètre agencé de façon à mesurer la fluorescence accumulée par les cellules vivantes; caractérisé en ce que le fluorimètre est disposé de manière à mesurer les modifications de fluorescence par une observation photométrique directe des cellules contenues dans le recipient de culture.

18. Appareil selon la revendication 17, dans lequel le fluorimètre comprend un appareil photographique ou une caméra vidéo, disposé de façon à effectuer une observation photométrique directe des cellules.

Patentansprüche

1. Verfahren zur Ermittlung der Lebensfähigkeit tierischer Zellen in einer bestimmten, in einem Kulturgefäß gebildeten Kulturumgebung, bestehend aus den Schritten, daß a) die Zellen in der Kulturumgebung bereitgestellt werden, b) die Zellen durch Perfusion eines Nährstoffes in die Umgebung gezüchtet werden, c) die Zellen mit einem fluorogenen Substrat kontaktiert werden, wodurch die lebenden Zellen einen charakteristischen Betrag an Fluoreszenz speichern, und d) Veränderungen in der Fluoreszenz als eine Anzeige der Lebensfähigkeit gemessen werden, dadurch gekennzeichnet, daß die Veränderungen in der Fluoreszenz durch eine direkte fotometrische Beobachtung der Zellen, während sie in der Umgebung innerhalb des Kulturgefäßes verbleiben, gemessen werden.

2. Verfahren zur Ermittlung der Sensitivität von Biopsiezellen gegenüber zytotoxischen Agenzien, bestehend aus den Schritten, daß a) die Zellen in einer in einem Kulturgefäß gebildeten Kulturumgebung bereitgestellt werden, b) die Zellen durch Perfusion eines Nährstoffes in die Umgebung gezüchtet werden, c) die Zellen mit einem fluorogenen Substrat kontaktiert werden, wodurch die lebenden Zellen einen charakteristischen Betrag an Fluoreszenz speichern, d) die Zellen mit dem zytotoxischen Agens kontaktiert werden und e) Veränderungen in der Fluoreszenz als Anzeige der Sensitivität der Zellen gegenüber dem zytotoxischen Agens gemessen werden, dadurch gekennzeichnet, daß die Veränderungen in der Fluoreszenz durch eine direkte fotometrische Beobachtung der Zellen, während sie in der Umgebung innerhalb des Gefäßes verbleiben, gemessen werden.

3. Verfahren nach Anspruch 2, bei dem die Biopsiezellen durch Aufspaltung einer Biopsieprobe in Fragmente und Herbeiführung einer Trennung zwischen irgendwelchem bösartigem Zellmaterial und normalem Zellmaterial durch Dekantieren aufbereitet werden.

4. Verfahren nach Anspruch 3, bei dem die Probe mechanisch in Fragmente aufgespalten wird.

5. Verfahren nach Anspruch 3 oder Anspruch 4, bei dem die Probe in Fragmente in der Größenordnung von 0,2 Kubikmillimeter aufgespalten wird.

6. Verfahren nach einem der Ansprüche 2 bis 5, bei dem die Zellen mit dem zytotoxischen Agens durch Perfusion kontaktiert werden.

7. Verfahren nach einem der vorhergehenden Ansprüche, bei dem die Veränderungen in der Fluoreszenz fotometrisch durch eine Foto- oder Videokamera aufgezeichnet werden.

8. Verfahren nach einem der vorgehenden Ansprüche, bei dem die fotometrische Beobachtung bei durch blaues monochromatisches Licht beleuchteter Kultur durchgeführt wird.

9. Verfahren nach Anspruch 7, bei dem die foto-

metrische Beobachtung eine Lichtfilterung von der Kultur mit einem Anpassungsblaufilter einschließt.

10. Verfahren nach einem der vorhergehenden Ansprüche, bei dem die direkte fotometrische Beobachtung der Zellen durch eine transparente Wandoberfläche des Gefäßes erfolgt.

11. Verfahren nach einem der vorhergehenden Ansprüche, bei dem die Perfusion mittels einer fluidpermeablen Membran stattfindet.

12. Verfahren nach Anspruch 11, bei dem die fluidpermeable Membran eine Porengröße von 5 bis 35 Mikrometer aufweist.

13. Verfahren nach Anspruch 11, bei dem die fluidpermeable Membran ein synthetisches Material umfaßt.

14. Verfahren nach Anspruch 11, bei dem die fluidpermeable Membran ein faseriges Zellulosematerial umfaßt.

15. Verfahren nach Anspruch 11, bei dem die fluidpermeable Membran ein polymeres Maschenwerk umfaßt.

16. Verfahren nach Anspruch 11, bei dem zwei einander gegenüberliegende Seiten des Gefäßes von fluidpermeablen Membranen gebildet sind, durch die die Perfusion stattfindet.

17. Vorrichtung, konstruiert und ausgebildet zur Durchführung des Verfahrens nach einem der vorhergehenden Ansprüche, bestehend aus a) einem Kulturgefäß, das durch eine fluidpermeable Perfusionsmembran in eine zellhaltige Zone und eine nährstoffhaltige Zone unterteilt ist, b) eine Quelle eines fluorogenen Substrats in Verbindung mit der zellhaltigen Zone und c) einem Fluorimeter zum Messen der von den lebenden Zellen gespeicherten Fluoreszenz, dadurch gekennzeichnet, daß das Fluorimeter eine Position zum Messen von Veränderungen in der Fluoreszenz durch eine direkte fotometrische Beobachtung der Zellen in dem Kulturgefäß besitzt.

18. Vorrichtung nach Anspruch 17, bei der das Fluorimeter eine Foto- oder Videokamera in einer Position für eine direkte fotometrische Beobachtung der Zellen umfaßt.

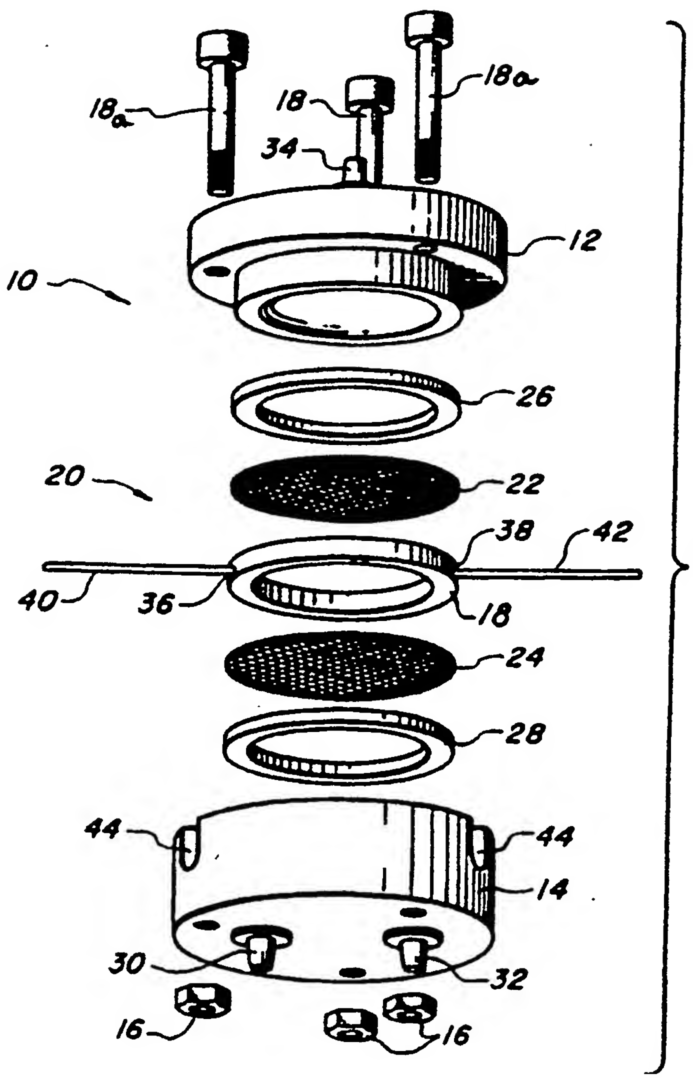


FIG. 1

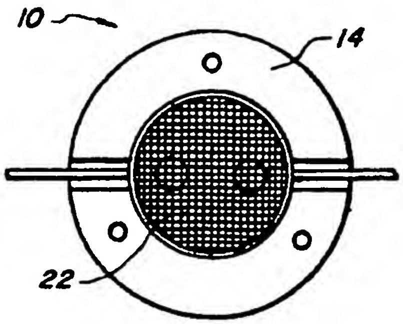


FIG. 3

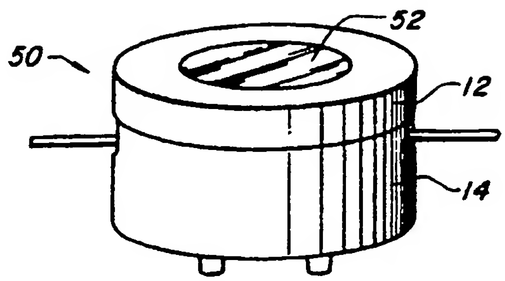


FIG. 4

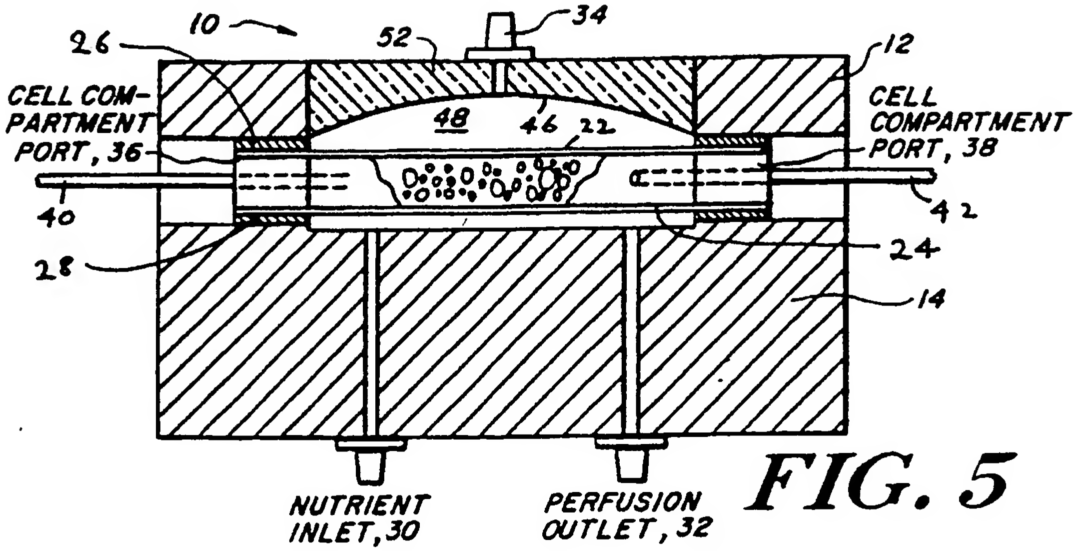


FIG. 5

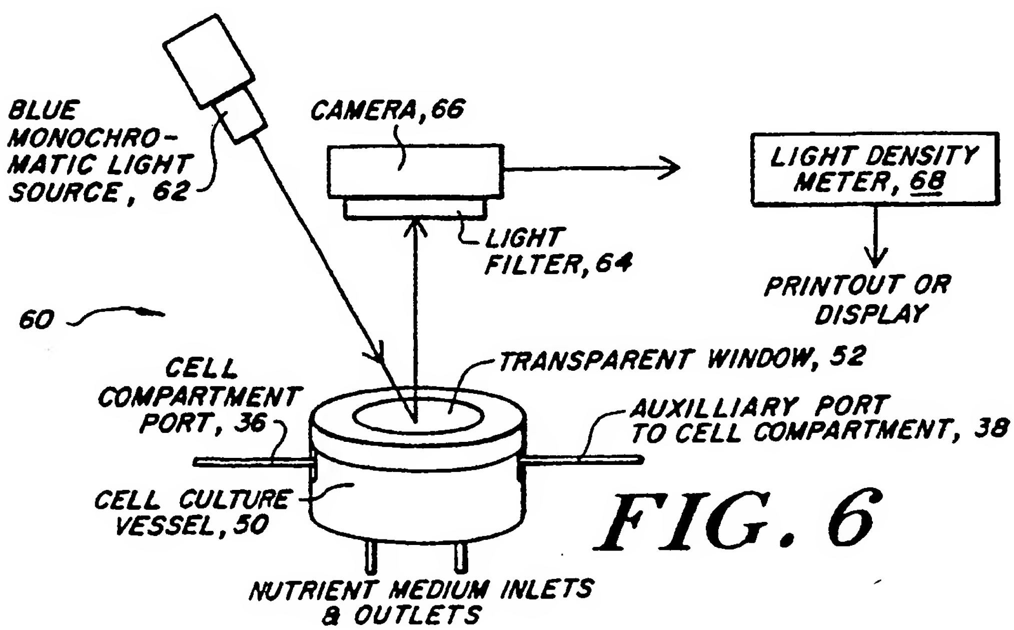
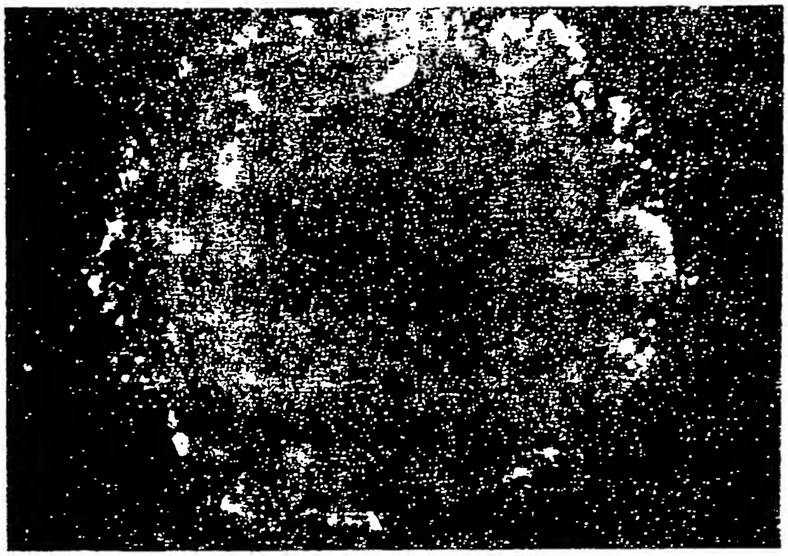
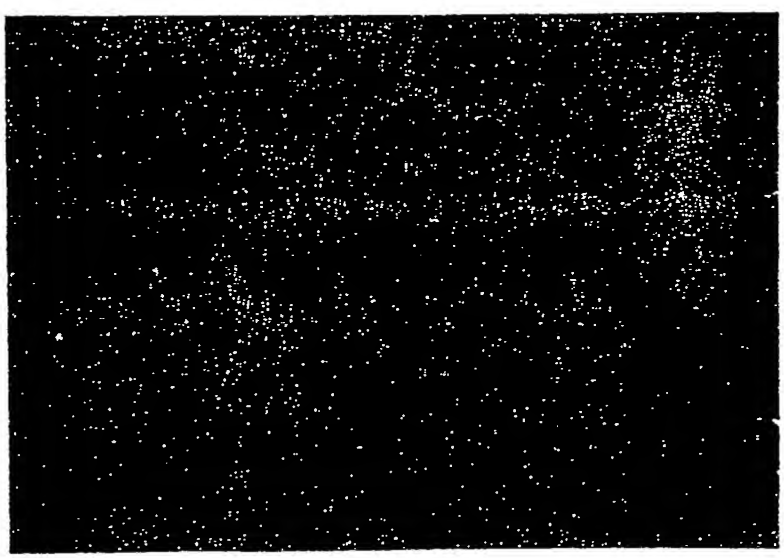


FIG. 6



CONTROL
TUMOR
WITHOUT
TREATMENT

FIG. 7A



TUMOR
TREATED
WITH
ADRIAMYCIN
FOR FIVE
DAYS

FIG. 7B